BIOSERVICE
SCIENTIFIC LABORATORIES GmbH

In vitro Mammalian Chromosome Aberration Test

in Chinese Hamster V79 cells

with WACKER BS 1701

Final Report

BSL BIOSERVICE Project No.: 001701

Sponsor:

Wacker-Chemie GmbH

Werk Burghausen

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Swift-Code: DEUTDEDBMUC

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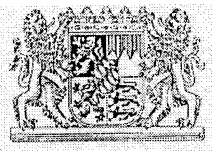

BAYERISCHES LANDESAMT FÜR ARBEITSSCHUTZ, ARBEITSMEDIZIN UND SICHERHEITSTECHNIK

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LfAS

GLP - B E S C H E I N I G U N G

Bescheinigung

Hiermit wird bestätigt, daß die Prüfeinrichtung(en)

BSL Bioservice Scientific Laboratories GmbH

in 82152 Planegg (Ort, Anschrift)

Behringstraße 6

der Firma BSL Bioservice Scientific Laboratories GmbH (Firma)

am 29./30. November 1999 (Datum)

von der für die Überwachung zuständigen Behörde über Einhaltung der Grundsätze der Guten Laborpraxis inspiziert worden ist (sind).

Es wird hiermit bestätigt, daß folgende Prüfungen in dieser Prüfeinrichtung nach den Grundsätzen der Guten Laborpraxis durchgeführt werden.

Certificate

It is hereby certified that the test facility(ies)

BSL Bioservice Scientific Laboratories GmbH

in 82152 Planegg (location, address)

Behringstraße 6

of Firma BSL Bioservice Scientific Laboratories GmbH (company name)

on 29./30. November 1999 (date)

was (were) inspected by the competent authority regarding compliance with the Principles of Good Laboratory Practice.

It is hereby certified that studies in this test facility are conducted in compliance with the Principles of Good Laboratory Practice.

Die Prüfungen von Stoffen und Zubereitungen betreffen folgende OECD-Prüfkategorie

Prüfkategorie 2: Prüfungen auf toxikologische Eigenschaften

Prüfkategorie 3: Prüfungen auf mutagene Eigenschaften (in vitro, in vivo)

Prüfkategorie 9: Sonstige Prüfungen; a) Mikrobiologische Sicherheitsprüfungen
b) Wirksamkeitsprüfungen an Zellkulturen

München, 04.08.2000
I.V.

Ritter
Leitender Gewerbedirektor

Contents

Preface

General Sponsor:	Wacker-Chemie GmbH Werk Burghausen Johannes-Hess-Strasse 24 D-84489 Burghausen, Germany
Monitor:	Dr. Axel Bosch
Testing Facility:	BSL BIOSERVICE Scientific Laboratories GmbH Behringstrasse 6 D-82152 Planegg/Munich
BSL BIOSERVICE - Project No.:	001701
Test Item:	WACKER BS 1701
Title:	*In vitro* Mammalian Chromosome Aberration Test in Chinese Hamster V79 cells with WACKER BS 1701

Project Staff

Study Director:	Dipl.-Biol. Uwe Hamann
Deputy Director of the Testing Facility:	Dr. Angela Lutterbach
Quality Assurance Unit:	Dr. Margarete Hoechst Dipl. Biol. Maike Führböter

Schedule

Date of Project Protocol:	October 26, 2000
Arrival of Test Item:	October 26, 2000
Start of Experiments:	November 02, 2000
End of Experiments:	February 06, 2001
Date of Draft Report:	February 08, 2001
Date of Final Report:	February 27, 2001

Project Staff Signatures

Study Director: Dipl.-Biol. Uwe Hamann

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Date: 22.02.2001

Deputy Director of the Testing Facility : Dr. Angela Lutterbach

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Date: 27.02.2001

Quality Assurance

This study was conducted to comply with:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anlage 1 ("Annex 1"), dated August 01, 1994 (BGBL. I, 1994, S. 1703).

OECD Principles of Good Laboratory Practice (as revised in 1997); OECD Environmental Health and Safety Publications; Series on Principles of Good Laboratory Practice and Compliance Monitoring - Number 1. Environment Directorate, Organisation for Economic Co-operation and Development, Paris 1998.

This study was assessed in compliance with the project protocol, the study plan and the Standard Operation Procedures of BSL BIOSERVICE. The study and/or the testing facility was periodically inspected by the Quality Assurance Unit and the dates and phases of the inspections are included in the report. These inspections and audits were carried out by the Quality Assurance Unit, personnel independent of staff involved in the study. The final report of the study will be audited. A Quality Assurance Statement, signed by the Quality Assurance, is included in the report.

Guidelines

This study followed the procedures indicated by the following internationally accepted guidelines and recommendations:

Ninth Addendum to OECD Guidelines for Testing of Chemicals, Section 4, No. 473, adopted 21st July, 1997, „*In vitro* Mammalian Chromosome Aberration Test"

EEC Directive 2000/32, L 136, Annex 4A, B 10, dated June 08, 2000.

EPA Health Effects Test Guidelines, OPPTS 870.5375 „In Vitro Mammalian Cytogenetics", EPA712-C-96-223, June 1996 (Public Draft)

Archiving

The following records will be stored in the scientific archives of BSL BIOSERVICE Scientific Laboratories GmbH according to the GLP-regulations:

a copy of the final report, the project protocol, the study plan and a documentation of all raw data generated during the conduct of the study (documentation forms as well as any other notes of raw data, microscopic slides, printouts of instruments and computers) and the correspondence with the sponsor concerning the project.

The microscopic slides and a sample of the test item (if left over) will be stored according to the period fixed by the GLP-regulations. Samples that are unstable may be disposed before that time. Unless otherwise agreed upon, remaining test item will be discarded three months after release of the report. No raw data or material relating to the study will be discarded without the sponsor's prior consent.

Statement of Compliance

BSL BIOSERVICE

Project-No.:	001701
Test Item:	WACKER BS 1701
Study Director:	Dipl.-Biol. Uwe Hamann
Title:	*In vitro* Mammalian Chromosome Aberration Test in Chinese Hamster V79 cells with WACKER BS 1701

This study performed in the testing facilities of BSL BIOSERVICE Scientific Laboratories GmbH was conducted in compliance with Good Laboratory Practice Regulations:

Chemikaliengesetz ("Chemicals Act") of the Federal Republic of Germany, Anlage 1 ("Annex 1"), dated August 01, 1994 (BGBL. I, 1994, S. 1703).

"OECD Principles of Good Laboratory Practice", as revised in 1997, Paris 1998.

There were no circumstances that may have affected the quality or integrity of the study.

Study Director: Dipl.-Biol. Uwe Hamann

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Date: 28.02.2001

Quality Assurance

BSL BIOSERVICE
Scientific Laboratories GmbH
Behringstr. 6, D-82152 Planegg

Statement

BSL BIOSERVICE

Project-No.:	001701
Test Item:	WACKER BS 1701
Study Director:	Dipl.-Biol. Uwe Hamann
Title:	*In vitro* Mammalian Chromosome Aberration Test in Chinese Hamster V79 cells with WACKER BS 1701

This report was audited by the Quality Assurance Unit and the conduct of this study was inspected on the following dates:

	Phases and Dates of QAU Inspections	Dates of Reports to the Study Director and Management
Audit: Project Protocol/ Study Plan	October 10, 2000	October 10, 2000
Experimental Phase Audit (Facility based Audit):	March 31, 2000	April 04, 2000
Draft Audit:	February 16, 2001	February 16, 2001
Final Audit:	February 28, 2001	February 28, 2001

This report reflects the raw data.

Head of Quality Assurance — Dr. Margarete Hoechst (or)
Dipl. Biol. Maike Führböter

Margarete Hoechst

Date: 28. 02. 2001

Summary

In order to investigate a possible potential of WACKER BS 1701 for its ability to induce structural chromosome aberrations in V79 cells of the Chinese hamster *in vitro* a chromosome aberration assay was carried out.

In two independent experiments the chromosomes were prepared 20 h after start of treatment with the test item. Experiment I was performed with and without S9 mix using a treatment interval of 4 h and a preparation interval of 20 h. Experiment II was performed only without metabolic activation with a treatment and preparation interval of 20 h. A prolonged preparation interval (28 h) was not performed since the test item showed slight toxic properties only in the 2nd experiment. Two parallel cultures were set up per test group. Per culture 100 metaphases were scored for structural chromosomal aberrations.

The following concentrations were evaluated :

Experiment I: 4 h treatment, 20 h preparation interval:
without S9 mix: 500, 2000, 5000 µg/ml
with S9 mix: 250, 2000, 5000 µg/ml

Experiment II: 20 h treatment and preparation interval:
without S9 mix: 50, 200, 500 µg/ml

In the 1st experiment the treatment of the cells with the test item did not lead to a relevant decrease of the relative mitotic index or of the cell density. Slight precipitation was observed at the highest concentration without metabolic activation of 5000.0 µg/ml. In the 2nd experiment a slight decrease of the relative mitotic index or of the cell density could be observed. Precipitation was observed, therefore the highest concentration for evaluation of metaphases was 500.0 µg/ml .

As compared to the corresponding solvent control no biologically relevant increase in aberrant cells was obtained with and without metabolic activation in both independent experiments.

No biologically relevant increase in the frequencies of polyploid metaphases was found after treatment with the test item if compared to the frequencies of the controls.

As positive controls reference mutagens (EMS, Ethylmethanesulfonate without S9 mix; CPA, Cyclophosphamide with S9 mix) were tested in parallel to the test item. They showed a distinct increase in cells with structural chromosome aberrations.

Conclusions

According to the reported data of this chromosome aberration study using V79 Chinese hamster cells it can be stated that in this study, the test item did not induce structural chromosome aberrations after metabolic activation.

Therefore, WACKER BS 1701 is considered to be non-mutagenic in this chromosome aberration test.

Objective

Introduction

The *in vitro* chromosomal aberration (CA) test is a mutagenicity test system for the detection of chromosomal aberration in Chinese hamster V79 cells.

Chromosome aberration assays detect the induction of chromosome breakage (clastogenesis). Although mutagenic substances produce structural chromosome aberrations by a variety of mechanisms, the endpoint is a discontinuity in the chromosomal DNA which is left unrejoined, or rejoined inaccurately, thus producing a mutated chromosome. Many of these changes will be lethal to the cell during the first few cell cycles after their induction but are used as indicators of the presence of non-lethal changes such as reciprocal translocations, inversions and small deletions. These more subtle changes may have important consequences in both germ and somatic cells. Chromosomal mutations and related events are the cause of many human genetic diseases and there is substantial evidence that these changes including oncogens and tumor suppressor genes are involved in carcinogenesis in humans and experimental systems. Chromosome aberrations are generally evaluated in first post treatment mitoses. The majority of chemical mutagens induced aberration of the chromatide type, but chromosome type aberrations also occur.

For treatment an asynchronous population of V79 cells in exponential growth should be used. A fixation time of around 20 hours after treatment is appropriate since the guidelines recommend fixation times of about 1.5-fold of the normal cell cycle and the normal cell cycle of the cells is 12-14 hours. If the test item causes extensive mitotic delay an additional later sampling time (28 h) should be included.

At least three concentrations of the test item should be used at fixation time 20 h. The highest concentration should be in the toxic range and should show a significant reduction in mitotic index or in degree of cell confluency (50% or greater). The lowest dose should be in the range of the negative control.

If an additional sampling time is carried out due to the highly toxic effects of the test item (delayed fixation time 28 hours) the same concentration range which induced a suitable degree of mitotic inhibition at the earlier fixation time, should be chosen.

Though the purpose of the assay is to detect structural chromosome aberrations, it is important to report polyploidy and/or endoreduplication when this is seen.

To validate the test, reference mutagens was tested in parallel to the test item.

Materials and Methods

Characterisation of the Test Item

The test item and the information concerning the test item were provided by the sponsor.

Name:	WACKER BS 1701
Chemical Description:	Alkylalkoxysilane
Batch No.:	KH 02343
CAS.-No.:	35435-21-3
Aggregate State at RT:	liquid
Colour:	colourless
Density (g/cm^3):	0.86 at 25°C
Structural Formula:	not provided
Purity:	98.53%
Analysis:	GC
Stability:	Pure: years Stable in aqueous suspension for at least 24 hours
Storage:	at room temperature, protected from light
Expiry Date:	November 2001
Safety Precautions:	Routine hygienic procedures will be sufficient to assure personnel health and safety.

The test item was prepared in cell culture medium and diluted prior to treatment.

Controls

Positive and negative controls were included.

Negative Controls

Solvent controls, consisting of solvent or vehicle alone and treated in the same way as the treatment groups were included. Concurrent negative and/or solvent controls were performed.

Positive Control

Without metabolic activation

Name	EMS; Ethylmethanesulfonate
Supplier	Merck-Schuchardt, D-85662 Hohenbrunn and SIGMA, D-82039 Deisenhofen
Catalogue no.	820774 (purity: > 98 %) and M 0880
Lot no.	S01417 642 and 99H0662
Dissolved in	nutrient medium
Final concentration	150 and 900µg/ml (1.21 and 7.28 mM)

The solution was prepared on the day of experiment.

The stability of the positive control substance in solution is proven by the mutagenic response in the expected range.

With metabolic activation

Name	CPA; Cyclophosphamide
Supplier	SIGMA, D-82039 Deisenhofen
Catalogue no.	C0768 (purity: at least 98 %)
Lot no.	087HO207
Dissolved in	nutrient medium
Final concentration	0.82 µg/ml (3 µM)

The stability of CPA at room temperature is good. At 25 °C only 3.5 % of its potency is lost after 24 hours (9).

Test System

The Cells

V79 cells *in vitro* are widely used to examine the ability of chemicals to induce cytogenetic changes and thus identify potential carcinogens or mutagens. These cells are chosen because of their relatively small number of chromosomes and because of the high proliferation rate (doubling time of the BSL BIOSERVICE V79 done in stock cultures: 13-14 h) and a high

plating efficiency of untreated cells (normally more than 50%). These facts are necessary for the appropriate performance of the study.

The V79 cells (supplied by GSF, D-80807 Neuherberg) are stored in liquid nitrogen in the cell bank of BSL BIOSERVICE, as large stock cultures allowing the repeated use of the same cell culture batch in experiments. Routine checking of mycoplasma infections as well as karyotype stability are carried out before freezing.

For the experiment thawed cultures were set up in 80 cm^2 plastic flasks (GREINER, D-72632 Frickenhausen) at 37° C in a 5.0% carbon dioxide atmosphere (95.0% air). Approximately 5 x 10^5 cells per flask were seeded in 15 ml of MEM (minimum essential medium; BIO-WHITTAKER, D-94501 Aidenbach) supplemented with 10% FCS (fetal calf serum; Seromed, D-12247 Berlin) and subcultures were made every 3-4 days.

Mammalian Microsomal Fraction S9 Mix

An advantage of using *in vitro* cell cultures is the accurate control of the concentration and exposure time of cells to the test item under study. However, due to the limited capacity of cells growing *in vitro* for metabolic activation of potential mutagens an exogenous metabolic activation system is necessary. Many substances only develop mutagenic potential when they are metabolised by the mammalian organism. Metabolic activation of substances can be achieved by supplementing the cell cultures with liver microsome preparations (S9 mix).

The S9 liver microsomal fraction (species: rat, strain: Wistar, sex: male, inducing agents: ß-Naphtoflavone and Phenobarbital) was prepared by BSL BIOSERVICE.

The following quality control determinations are performed by BSL BIOSERVICE:

a) Biological activity in the *Salmonella typhimurium* assay
b) Sterility Test

A stock of the supernatant containing the microsomes is frozen in ampoules of 2 and 5 ml and stored at -80°C or –<130°C (vapour phase liquid nitrogen). Small numbers of ampoules may be kept at -20° C for only one week before use.

The protein concentration in the S9 preparation (Lot: 101299) was 33.4 mg/ml.

S9 Mix

An appropriate quantity of S9 supernatant was thawed and mixed with S9 cofactor solution. The final protein concentration of the S9 mix in the cultures was 0.75 mg/ml. The cofactor supplemented post-mitochondrial fraction contained the following supplements:

8 mM $MgCl_2$

33 mM KCl

5 mM Glucose-6-Phosphate

4 mM NADP

in 100 mM sodium-phosphate-buffer, pH 7.4

During the experiment the S9 mix was stored in an ice bath. The S9 mix preparations were performed according to Ames et al. (1)

Pre-Experiment for Toxicity

No pre-experiment was carried out.

Dose Selection

Several concentrations of the test item extract were chosen to be applied in the chromosomal aberration assay. The highest concentration used in the experiments was 5000.0 µg/ml.

The treatment interval was 4 h with and without metabolic activation and 20 h without metabolic activation. Per concentration duplicate cultures were used.

No toxic effects (reduction of the relative cell density, low metaphase number, partially combined with poor metaphase quality), which restrained the evaluation of metaphases were seen in experiment I.

Experiment II was set up with several concentrations for 20 h treatment without metabolic activation. In the 2nd experiment precipitation and slight toxic effects were observed, therefore the highest concentration for evaluation of metaphases were 500.0 µg/ml.

The following concentrations were chosen for evaluation:

Experiment I: 4 h treatment, 20 h preparation interval:

Without S9 mix: 500, 2000 and 5000 µg/ml
With S9 mix: 250, 2000 and 5000 µg/ml

Experiment II: 20 h treatment and preparation interval:
without S9 mix: 50, 200, 500 µg/ml

Experimental Performance

Seeding of the Cultures

Three or four days old stock cultures (in exponential growth) more than 50% confluent were trypsinised at 37 °C for 5 minutes by adding a trypsine solution (Life Technologies, D-76344 Eggenstein) in Ca-Mg-free salt solution. By adding complete culture medium the enzymatic treatment was stopped. A single cell suspension was prepared. The trypsine concentration for all subculturing steps was 0.2 %.

The cells were rinsed with Ca-Mg-free PBS solution prior to the trypsine treatment.

The cells were seeded into Quadriperm dishes (HERAEUS, D-63450 Hanau) which contain microscopic slides (at least 2 chambers per dish and

test group). Into each chamber 1 x 10^4 - 4 x 10^4 cells were seeded with regard to preparation interval. The medium was minimum essential medium supplemented with 10 % FCS.

Treatment:

Two days after seeding of the cells, the culture medium was replaced with serum-free medium containing the test item and 50 µl/ml S9 mix. Additional negative and positive controls were performed with exogenic metabolic activation.

Exposure time 4 hours (with and without S9 mix):

4 h after the treatment the cultures were washed twice with PBS and then the cells were cultured in complete medium for the remaining culture time.

Exposure time 20 hours (without S9 mix; second experiment):

Two days after seeding of the cells the culture medium was replaced with complete medium (10% FCS) containing the test item. This medium was not changed until preparation of the cells. Additional negative and positive controls were performed.

Preparation of the Cultures

17.5 h **(4 h and 20 h treatment interval)** after the start of the treatment colcemide was added to the cultures (0.2 µg/ml culture medium). 2.5 h later, the cells were treated on the slides in the chambers with hypotonic solution (0.4 % KCl) for 20 min at 37 °C. After incubation in the hypotonic solution the cells were fixed with 3 + 1 methanol + glacial acetic acid. All the steps were carried out on precision hot plates. After fixation the cells were stained with Giemsa (MERCK, D-64293 Darmstadt).

Analysis of Metaphase Cells

All slides, including those of positive and negative controls were independently coded before microscopic analysis. Evaluation of the cultures was performed [according to standard protocol of the "Arbeitsgruppe der Industrie, Cytogenetik" (3)] using OLYMPUS microscopes with 100x oil immersion objectives. As structural chromosomal aberrations breaks, fragments, deletions, exchanges and chromosomal desintegrations were recorded. Gaps were recorded as well but not included in the calculation of the aberration rates. The definition of a gap is as follows: an achromatic region (occurring in one or both chromatids) independent of its width. The remaining visible chromosome regions should not be dislocated either longitudinally or laterally. At least 200 well spread metaphases per concentration and control were scored for cytogenetic damage. The cells scored contained 22 ± 1 centromeres. To describe a cytotoxic effect the mitotic index (% cells in mitosis) was determined by counting the number of mitotic cells in 1000 cells. Additionally the number of polyploid cells was scored. Polyploid means a near tetraploid karyotype in the case of this aneuploid cell line.

Analysis of Relative Cell Density

As an additional parameter for cytotoxic effects of the test item the relative cell density was calculated as the mean of twenty cell counts per test group (cells within the visual field at a 400-fold magnification).

Data Recording

The data generated were recorded in the raw data file. The results are presented in tables, including experimental groups with the test item, negative and positive controls. The experimental unit is the cell and therefore, the percentage of cells with structural aberration is evaluated. Different types of chromosome aberrations are listed with their numbers of frequencies for experimental and control groups. Gaps are recorded separately and reported but generally not included in the aberration frequency. Concurrent measures of cytotoxicity are also recorded.

Acceptability of the Assay

The chromosomal aberration assay is considered acceptable if it meets the following criteria:

- the number of aberrations found in the negative control ranges between 0.00 % and 4.50 %,
- the positive control substance should produce biologically relevant increases in the number of cells with structural chromosome aberrations.

Evaluation of Results

There are several criteria for determining a positive result:

- dose-related increase in the number of cells with aberration,
- biologically relevant positive response for at least one of the test points,

According to the new OECD guidelines, the biological relevance of the results has been the criterion for the interpretation of results, a statistical evaluation of the results was not regarded as necessary.

A test item is considered to be negative if there is no biologically relevant increase in the percentages of aberrant cells above concurrent control levels.

Deviation to Project Protocol

Concerning: Positive Control *Without metabolic activation*

Before:	
Name:	EMS; Ethylmethanesulfonate
Supplier:	Merck-Schuchardt, D-85662 Hohenbrunn
Catalogue no.:	820774 (purity: > 98 %)
Final concentration:	4.85 mM

New:	
Name	EMS; Ethylmethanesulfonate
Supplier	Merck-Schuchardt, D-85662 Hohenbrunn and SIGMA, D-82039 Deisenhofen
Catalogue no.	820774 (purity: > 98 %) and M 0880
Lot no.	S01417 642 and 99H0662
Final concentration:	150 and 900 µg/ml (1.21 and 7.28 mM)

Reasons for alteration:
Updating the experimental conditions and EMS is not longer available from Merck-Schuchardt

These deviations did not influence the quality and integrity of the study.

Results

Tables of Results

Abbreviations

The following abbreviations are used in the tables with structural chromosomal aberrations:

g = gap and **ig** = iso-gap; gaps are achromatic lesions of chromatid or chromosome type where no dislocation of chromosomal material is visible (independent of the size of the achromatic region).

b = break and **ib** = iso-break

f = fragment; **if** = iso-fragment

d = deletion; **id** = iso-deletion

ma = multiple aberration is defined as a metaphase containing more than 4 events [excluding gaps]; only exchanges are recorded additionally in these cells)

ex = chromatid type exchange

cx = chromosome type exchange

cd = chromosomal disintegration (pulverisation)

Table 1: Number of Polyploid Cells, Mitotic Index and Relative Cell Density. Without and with S9 mix, **4 h** treatment, **20 h** fixation period

Dose group	Concentration (µg/ml)	Polyploid Cells *			Mitotic Index***				Relative Cell Density (%)****			
		Culture 1	Culture 2	mean	Culture 1	Culture 2	mean	Relative** Mitotic Index (%)	Culture 1	Culture 2	mean	Relative Cell Density (%)
without S9 mix												
K-[1]		6.0	6.0	6.0	87	134	110.5	100	44	41	42.5	100
P-[2] **(EMS)**	900.0	5.0	9.0	7.0	103	125	114.0	103	47	36	41.5	98
1-	500	0.0	3.0	1.5	140	170	155.0	140	47	56	51.5	121
2-	2000	0.0	2.0	1.0	134	122	128.0	116	73	52	62.5	147
3-	5000	3.0	5.0	4.0	88	101	94.5	86	58	40	49.0	113
with S9 mix												
K+[1]		3.0	4.0	3.5	82	102	92.0	100	94	72	83.0	100
P+[2] **(CPA)**	0.82	6.0	4.0	5.0	114	117	115.5	126	45	67	56.0	67
1+	250	5.0	2.0	3.5	84	87	85.5	93	70	87	78.5	95
2+	2000	5.0	2.0	3.5	76	98	87.0	95	58	61	59.5	72
3+	5000	1.0	3.0	2.0	97	56	76.5	83	74	76	75.0	90

*: The number of polyploid cells was determined in 100 cells per culture of each test group.
**: The relative values of the mitotic index are related to the solvent controls.
***: The mitotic index was determined in 1000 cells per culture of each test group.
****: The cell density was calculated as the mean of ten cell counts per slide (cells within the visual field at a 400-fold magnification)
1: Negative Control (Culture Medium)
2: Positive Control

Table 2: Structural Chromosomal Aberrations, with metabolic activation. With S9 mix, **4 h** treatment, **20 h** fixation period.

Dose Group	Concentration (µg/ml)		Cells Scored	Aberrant Cells Incl. Gaps	Aberrant Cells excl. gaps	Gaps		Types of Aberrations Found									
								Chromatid Type				Chromosome Type				Other	
						g	ig	b	f	d	ex	ib	if	id	cx	ma	Cd
		culture 1	100	5	3	2	0	2	1	0	0	0	0	0	0	0	0
K+[1]		culture 2	100	2	2	0	0	0	0	0	2	0	0	0	0	0	0
		Total	**200**	**7**	**5**	**2**	**0**	**2**	**1**	**0**	**2**	**0**	**0**	**0**	**0**	**0**	**0**
		culture 1	100	11	10	1	0	3	0	0	8	0	0	0	0	0	0
P+[2]	0.82	culture 2	100	17	15	3	0	3	3	1	9	0	0	0	0	0	0
(CPA)		**Total**	**200**	**28**	**25**	**4**	**0**	**6**	**3**	**1**	**17**	**0**	**0**	**0**	**0**	**0**	**0**
		culture 1	100	4	1	3	0	0	0	0	1	0	0	0	0	0	0
1+	250	culture 2	100	2	0	1	1	0	0	0	0	0	0	0	0	0	0
		Total	**200**	**6**	**1**	**4**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
		culture 1	100	2	1	1	0	0	0	0	1	0	0	0	0	0	0
2+	2000	culture 2	100	3	2	1	0	2	0	0	0	0	0	0	0	0	0
		Total	**200**	**5**	**3**	**2**	**0**	**2**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
		culture 1	100	1	1	0	0	0	0	0	1	0	0	0	0	0	0
3+	5000	culture 2	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		Total	**200**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**

1: Negative Control (Culture Medium)
2: Positive Control

Table 3: Structural Chromosomal Aberrations, without metabolic activation. Without S9 mix, **4 h** treatment, **20 h** fixation period

Dose Group	Concentration (µg/ml)		Cells Scored	Aberrant Cells Incl. Gaps	Aberrant Cells Excl. Gaps	Gaps		Types of Aberrations Found									
								Chromatid Type			Chromosome Type					Other	
						g	ig	b	f	d	Ex	ib	if	id	cx	ma	cd
K-[1]		culture 1	100	1	0	1	0	0	0	0	0	0	0	0	0	0	0
		culture 2	100	2	0	2	0	0	0	0	0	0	0	0	0	0	0
		Total	**200**	**3**	**0**	**3**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
P-[2] (EMS)	900.0	culture 1	100	19	17	4	0	4	2	0	15	0	0	0	0	0	0
		culture 2	100	20	20	0	0	10	3	1	6	1	0	0	4	0	0
		Total	**200**	**39**	**37**	**4**	**0**	**14**	**5**	**1**	**21**	**1**	**0**	**0**	**4**	**0**	**0**
1-	500	culture 1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		culture 2	100	4	4	0	0	1	2	1	0	0	0	0	0	0	0
		Total	**200**	**4**	**4**	**0**	**0**	**1**	**2**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
2-	2000	culture 1	100	1	1	0	0	1	0	0	0	0	0	0	0	0	0
		culture 2	100	1	1	0	0	0	0	0	1	0	0	0	0	0	0
		Total	**200**	**2**	**2**	**1**	**0**	**1**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**
3-	5000	culture 1	100	3	2	2	0	1	0	1	0	0	0	0	0	0	0
		culture 2	100	3	3	0	0	1	0	0	2	0	0	0	0	0	0
		Total	**200**	**6**	**5**	**2**	**0**	**2**	**0**	**1**	**2**	**0**	**0**	**0**	**0**	**0**	**0**

1: Negative Control (Culture Medium)
2: Positive Control

Table 4: Number of Polyploid Cells, Mitotic Index and Relative Cell Density. Without S9 mix, **20 h** treatment and fixation period

Dose group	Concentration (µg/ml)	Polyploid Cells *			Mitotic Index***				Relative Cell Density (%)****			
		Culture 1	Culture 2	mean	Culture 1	Culture 2	mean	Relative** Mitotic Index (%)	Culture 1	Culture 2	mean	Relative Cell Density (%)
without S9 mix												
K-[1]		4.0	7.0	3.5	134	142	138.0	100	110	111	110.5	100
P-[2] **(EMS)**	150	1.0	4.0	2.5	55	85	70.0	51	79	72	75.5	68
1-	50	6.0	3.0	4.5	126	124	125.0	91	120	135	127.5	115
2-	200	0.0	3.0	1.5	109	126	117.5	85	115	116	115.5	105
3-	500	4.0	2.0	3.0	48	32	40.0	29	73	50	61.5	55

*: The number of polyploid cells was determined in 100 cells per culture of each test group.
**: The relative values of the mitotic index are related to the solvent controls.
***: The mitotic index was determined in 1000 cells per culture of each test group.
****: The cell density was calculated as the mean of ten cell counts per slide (cells within the visual field at a 400-fold magnification)
1: Negative Control (Culture Medium)
2: Positive Control

Table 5: Structural Chromosomal Aberrations, with metabolic activation. With S9 mix, **20 h** treatment and fixation period.

Dose Group	Concentration (µg/ml)		Cells Scored	Aberrant Cells Incl. Gaps	Aberrant Cells excl. gaps	Gaps		Types of Aberrations Found								
								Chromatid Type			Chromosome Type				Other	
						g	ig	b	f	d	ex	ib	if	id	cx	ma	Cd
K[1]		culture 1	100	1	0	0	0	0	2	0	0	0	0	0	0	0	0
		culture 2	100	3	2	1	0	1	2	0	0	0	0	0	0	0	0
		Total	**200**	**4**	**2**	**1**	**0**	**1**	**4**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
P[2] (EMS)	150	culture 1	100	24	24	7	0	11	3	2	18	1	0	0	1	0	0
		culture 2	100	22	21	4	5	6	3	0	17	0	0	0	1	0	0
		Total	**200**	**46**	**45**	**11**	**5**	**17**	**6**	**2**	**35**	**1**	**0**	**0**	**2**	**0**	**0**
1-	50	culture 1	100	2	1	1	0	0	1	0	0	0	0	0	0	0	0
		culture 2	100	4	3	2	0	2	0	1	0	0	0	0	0	0	0
		Total	**200**	**6**	**4**	**3**	**0**	**2**	**1**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
3-	200	culture 1	100	5	4	1	0	3	1	0	0	0	0	0	0	0	0
		culture 2	100	1	1	0	0	1	0	0	0	0	0	0	0	0	0
		Total	**200**	**6**	**5**	**1**	**0**	**4**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**
4-	500	culture 1	100	0	0	0	0	0	0	0	0	0	0	0	0	0	0
		culture 2	100	1	0	0	0	1	0	0	0	0	0	0	0	0	0
		Total	**200**	**1**	**0**	**0**	**0**	**1**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**	**0**

1: Negative Control (Culture Medium)
2: Positive Control

Summary of Aberration Rates

Table 6:

Experiment I:

		S9 mix	treatment	fixation	mean % aberrant cells*	
	µg/ml		time	interval	incl. gaps	excl. gaps
K-	Culture Med.	-	4 h	20 h	1.5	0.0
P-	Pos. Control EMS	-	4 h	20 h	19.5	18.5
1-	500.0	-	4 h	20 h	2.0	2.0
2-	2000.0	-	4 h	20 h	1.0	1.0
3-	5000.0	-	4 h	20 h	3.0	2.5
K+	Culture Med.	+	4 h	20 h	3.5	2.5
P+	Pos. Control CPA	+	4 h	20 h	14.0	12.5
1+	250.0	+	4 h	20 h	3.0	0.5
2+	2000.0	+	4 h	20 h	2.5	1.5
3+	5000.0	+	4 h	20 h	0.5	0.5

* 200 cells evaluated for each concentration

Table 7:

Experiment II:

	µg/ml	S9 mix	treatment time	fixation interval	mean % aberrant cells* incl. gaps	excl. gaps
K-	Culture Med.	-	20 h	20 h	2.0	1.0
P-	Pos. Control EMS	-	20 h	20 h	23.0	22.5
1-	50.0	-	20 h	20 h	3.0	2.0
2-	200.0	-	20 h	20 h	3.0	2.5
3-	500.0	-	20 h	20 h	0.5	0.0

* 200 cells evaluated for each concentration

Discussion

The test item WACKER BS 1701 was investigated for a possible potential to induce structural chromosomal aberrations in V79 cells of the Chinese hamster *in vitro* in the absence and presence of metabolic activation by S9.

In two independent experiments the chromosomes were prepared 20 h after start of treatment with the test item. Experiment I was performed with and without S9 mix using a treatment interval of 4 h and a preparation interval of 20 h. Experiment II was performed only without metabolic activation with a treatment and preparation interval of 20 h. A prolonged preparation interval (28 h) was not performed since the test item showed slight toxic properties only in the 2nd experiment. Two parallel cultures were set up per test group. Per culture 100 metaphases were scored for structural chromosomal aberrations.

The following concentrations were evaluated:

Experiment I: 4 h treatment, 20 h preparation interval:
with S9 mix: 250, 2000, 5000 µg/ml
without S9 mix: 500, 2000, 5000 µg/ml

Experiment II: 20 h treatment and preparation interval:
without S9 mix: 50, 200, 500 µg/ml

Several concentrations of the test item were chosen to be applied in the chromosomal aberration assay. The highest concentration used in the experiments was 5000.0 µg/ml.

For evaluation 250, 2000 and 5000 µg/ml were selected in experiment I with and 500, 2000, 5000 µg/ml without S9 mix. No relevant reduction of the mitotic index and in cell density was observed with and without metabolic activation.

In the 2nd experiment (20 h treatment) without S9 mix precipitation was observed, therefore the highest concentration for evaluation of metaphases was 500.0 µg/ml. A slight decrease of the relative mitotic index or of the cell density could be observed. 50, 200 and 500 µg/ml were selected for evaluation.

In experiment I with and without metabolic activation the test item did not increase the frequency of the cells with aberrations. The aberration rates of the cells after treatment with the test item in experiment I (without S9 mix: 1.0-2.5%; with S9 mix: 0.5-1.5%) were near to the range of the negative control values (without S9 mix: 0.0 %, with S9 mix: 2.5 %).

Also in experiment II (performed only without S9 mix, continuous treatment) the aberration rates of the cells after treatment with the test item (0.0-2.5%) were near to the range of the negative control values (1.0%).

Additionally, all values obtained after treatment with the test item were within the range of our historical negative control data: 0.0 - 4.5% in both experiments with and without metabolic activation .

Table 1 and 4 show the occurrence of polyploid metaphases. No biologically relevant increase in the rate of polyploid metaphases was found after treatment with the test item.

EMS (150 and 900 µg/ml) and CPA (0.82 µg/ml) were used as positive controls. They showed a distinct and biologically relevant increase of cells with structural chromosome aberrations above our historical control level.

Conclusion

In conclusion, it can be stated that during the described chromosomal aberration test and under the experimental conditions reported, the test item did not induce structural chromosomal aberrations in the V79 Chinese hamster cell line.

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